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Environment has a Stronger Effect than Host Plant Genotype in Shaping Spring *Brassica napus* Seed Microbiomes

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ABSTRACT

Seed are reproductive units that transfer genetic information to the next generation and harbor microbial communities that may interact with a host plant at all stages of its development. Here, we assessed the effect of the environment and plant genotype on the seed microbiome of eight spring *Brassica napus* lines harvested from four site years in Saskatchewan, Canada: one location each in 2016 and 2017 and two additional locations in 2017. Seed microbiomes were characterized using high-throughput amplicon sequencing of the bacterial 16S ribosomal RNA and fungal internal transcribed spacer regions. Our results revealed that microbial communities were predominantly shaped by the environment, with location explaining 34% of bacterial and 43% of fungal total variance. Meanwhile, genotype had a smaller effect, accounting for only 9% of bacterial and 13% of fungal variance. The seed microbiome of *B. napus* predominantly contained members of

Enterobacteriales and Pseudomonadales bacterial orders as well as Pleosporales and Capnoidiales fungal orders. Additionally, common taxa, including Enterobacteriales, Pseudomonadales, Micrococcales, Sphingomonadales, Pleosporales, Capnoidiales, Tremellales, Filobasidiales, and Sporidiobolales, were detected across all site years. Our results demonstrate that the environment plays a dominant role in shaping spring *B. napus* seed microbiomes, with more subtle contributions related to host plant genotype. Information collected in this study may contribute to the development of novel and sustainable breeding and agricultural strategies that consider microorganisms carried by seed.

Keywords: agriculture, canola, crop, environment, host genotype, microbiome, seed microbiome

The plant microbiome contributes a significant level of genetic information that influences plant fitness (Sessitsch and Mitter 2015; Zilber-Rosenberg and Rosenberg 2008). Thus, microbial communities can be either beneficial or deleterious for plant growth, development, and function, thereby affecting productivity and yield of agricultural crops (Andreote and Pereira e Silva 2017; Tosi et al. 2020). Each plant compartment, including reproductive organs such as flowers (Arunkumar et al. 2019), fruit (Droby and Wisniewski 2018), and seed (Nelson 2018), provides resources and habitat for a huge number of microbes, which colonize external and internal

plant tissues. Seed not only represent the starting point for a broad variety of plant species but also store and carry microbes that will continue to interact with the plant throughout its life cycle (Gopal and Gupta 2016). Thus, a plant's first microbial inoculum comes from the seed.

Climate change, ecosystem degradation, and biodiversity loss significantly affect agriculture and food security; looking for sustainable strategies and practices is essential to ensure the global nutritional demand (Toju et al. 2018). Exploring plant microbiome functions is one approach that can mitigate adverse effects caused by biotic and abiotic stresses affecting plant fitness (Orozco-Mosqueda et al. 2018; Shinwari et al. 2019). Microbial communities associated with seed are reported to suppress disease (Jack and Nelson 2018; Khalaf and Raizada 2018; Links et al. 2014) and directly promote plant growth (Bergna et al. 2018; Walitang et al. 2017) in cereals, fruit, vegetables, and other crops, which reveals their potential application in sustainable crop production. There is a paucity of information known about seed-associated microbiota, especially about their composition, dynamics, and assemblage (Shade et al. 2017; Wassermann et al. 2019).

Rapeseed (*Brassica napus*), soybean (*Glycine max*), and palm (*Phoenix dactylifera*) are the main sources of vegetable oil in the world (Liu et al. 2018). Low-erucic and low-glucosinolate edible

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rapeseed varieties are known as “canola” (Canola Council of Canada 2020). Canola is considered a staple crop in the Canadian Prairies and crucial for global food security. Its use is not limited to human consumption; canola meal is utilized in animal feeds (Good et al. 2017) and as a feedstock for biodiesel production (Ge et al. 2018). Depending on the flowering time and local adaptation, canola cultivars grow during spring or winter seasons and can be annual or biannual (Schiessl et al. 2017). Production in Western Canada is based on spring canola, with the province of Saskatchewan the largest supplier, marketing approximately 11 million metric tons from 4,955,000 ha harvested in 2018 (Statistics Canada 2020). Canola-producing areas are found in different soil and climatic zones, which represent a broad range of environments and ecological habitats (Government of Saskatchewan 2019). Despite the agricultural and nutritional importance of this oilseed, there are few studies regarding its microbiome. In addition, the few reports available mainly focused on the canola root and rhizosphere microbiomes (Bazghaleh et al. 2020; Cordero et al. 2020; Floc’h et al. 2020; Lay et al. 2018; Monreal et al. 2018), whereas the seed microbiome is poorly understood.

In agricultural crops, assembly of the seed microbiome has been linked to host genotype (Adam et al. 2018; Raj et al. 2019), field management practices (Klaedtke et al. 2016), harvesting year (Rochefort et al. 2019), or anthropogenic pollutants (Truyens et al. 2016). Nevertheless, the relative contribution of environmental factors versus host genotype is mostly unknown. In a previous study (Moreira et al. 2021), we profiled the seed microbiome of wheat (*Triticum aestivum*), canola (*B. napus*), and lentil (*Lens culinaris*) lines across two generations. We found that crop, genotype, and the environment were key drivers of the seed microbiome assemblage. To better understand the contribution of differences in environment, we profiled eight genetically different spring *B. napus* lines harvested from different locations in Saskatchewan, Canada. We hypothesized that the host genotype (i.e., line) would influence the *B. napus* seed microbiome structure and diversity. We further hypothesized that the collective influences of the environment (i.e., all sources of variation that are not genetic such as location and harvesting year), would have a stronger influence than genotype on the seed microbiome. To test these hypotheses, 16S ribosomal RNA (rRNA) and internal transcribed spacer (ITS) amplicon libraries from seed samples collected in four site years (one location in 2016 and 2017 and two additional locations in 2017) were prepared and sequenced. Understanding how environmental and genetic factors

influence the assembly of the microbiota carried by seed could provide insights into the recruitment and transmission of microbial communities in plants. This understanding is essential for implementing novel and sustainable crop breeding and agricultural management practices.

MATERIALS AND METHODS

Lines and experimental design. *B. napus* seed used in this study were harvested from four site years in Saskatchewan, Canada: one location (Saskatoon) in 2016 and 2017 and two additional locations (Melfort and Scott) in 2017. These three sites span a large portion of the local canola-growing regions and represent the different conditions under which canola is grown. Fields were located in the Dark Brown Soil Zone (Saskatoon, Scott; Typic Boroll) and in the Black Soil Zone (Melfort; Udic Boroll) (Table 1; Supplementary Fig. S1). At all field sites, *B. napus* lines were planted in a randomized complete block design experiment (plots of 6 by 2 m), with three replicates. Eight lines from a nested association mapping (NAM) population were selected to represent diversity within the set (Bazghaleh et al. 2020; Taye et al. 2020): NAM 0, NAM 13, NAM 17, NAM32, NAM 37, NAM 43, NAM 72, and NAM 94. Seed planted at all fields came from the same source and were produced in hoop tents by Agriculture and Agri-Food Canada (Saskatoon, SK, Canada). All seed samples analyzed in this study were stored in paper bags at 21°C (room temperature) until analysis. DNA extraction of seed samples collected from each site year was carried out approximately 8 months after harvesting. Library preparation and Illumina sequencing were done in two batches for each gene. Samples from Saskatoon 2016, Saskatoon 2017, and Melfort 2017 comprised the first batch. Scott 2017 were processed separately due to a delayed harvest (Table 1; Supplementary Fig. S1) and subsequent delays in obtaining the seed.

DNA extraction and high-throughput amplicon sequencing. A modified protocol previously described by Rastogi et al. (2010) and Martins et al. (2013) was applied for DNA extraction of the seed epiphytic microbial communities. From each plot harvested, 5 g of seed was immersed in 25 ml of buffered wash solution containing 0.024% Triton, 10 mM EDTA, and 20 mM Tris-HCl in a 250-ml Erlenmeyer flask and shaken (150 rpm) for 15 min at room temperature. A 0.22-µm membrane (Pall, Mexico) was used to filter the solution followed by DNA extraction with the DNeasy PowerWater

TABLE 1
Saskatchewan, Canada field sites

Location	Year	GPS coordinates	Soil ^a	Seeding	Harvesting	Precip (mm) ^b	Temp (°C) ^c
Saskatoon	2016	52°10'52.918" N, 106°30'10.587" W	Dark Brown Chernozem (Typic Boroll), clay, loam texture, pH 7.5, OM 5.1%	27 May	10–22 September	195.3	16.5
	2017	52°10'59.336" N, 106°30'53.654" W	Dark Brown Chernozem (Typic Boroll), clay, loam texture, pH 7.5, OM 5.1%	28–29 May	5–28 August	81.6	17.5
Melfort	2017	52°49'9.599" N, 104°35'46.852" W	Black Chernozem (Udic Boroll), silty clay texture, pH 6.4, OM 8.2% ^d	19 May	9 September	92.5	16.6
Scott	2017	52°21'55.332" N, 108°52'32.555" W	Dark Brown Chernozem (Typic Boroll), loam texture, pH 5.7, OM 5.8% ^e	20 June	17 October	106.2	13.7

^a OM = organic matter.

^b Precipitation during growing season.

^c Mean temperature.

^d Foster et al. (2014).

^e Bedard-Haughn et al. (2013) and Arcand et al. (2016).

Kit (Qiagen, Hilden, Germany) and quantification in a Qubit Fluorometer (Invitrogen, Carlsbad, CA, U.S.A.). Bacterial 16S rRNA and fungal ITS regions were amplified using the primer sets with Illumina adapters 342F/806R (Mori et al. 2014) and ITS1-F_KYO1/ITS2-R_KYO2 (Toju et al. 2012), respectively. PCR assays were carried out using DreamTaq Hot Start DNA Polymerase (Thermo Scientific, Carlsbad, CA, U.S.A.) for 16S rRNA, and HotStarTaq Master Mix Kit (Qiagen) for ITS amplifications in a T100 Thermal cycler (Bio-Rad, Hercules, CA, U.S.A.) (Moreira et al. 2021). NucleoMag magnetic beads (NucleoMag NGS Clean-up and size select; Macherey-Nagel, Düren, Germany) were used to purify PCR products followed by barcoding with Nextera XT Index Kit v2 (Illumina Inc., San Diego, CA, U.S.A.) using KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, U.S.A.). Libraries were sequenced in an Illumina MiSeq platform with the MiSeq Reagent Kit v3 (600 cycles) for bacteria and MiSeq Reagent Kit v2 (500 cycles) for fungi. Negative controls were included in the library preparation.

Bioinformatics and statistical analysis. Cutadapt v.2.1 was used for removing primers (Martin 2011). Sequences were processed in QIIME2 v.2019.10 (Bolyen et al. 2019), quality filtered, and assigned to amplicon sequence variants (ASVs) in Deblur (bacteria) (Amir et al. 2017) and DADA2 (fungi) (Callahan et al. 2016), in accordance with a standardized protocol developed for soil, rhizosphere, root, leaf, and seed microbiome analyses (Bazghaleh et al. 2020). A 342F/806R-trained V3/V4 SILVA database v.132 (Quast et al. 2013) and UNITE database v.8.0. (Abarenkov et al. 2010) were used to classify bacterial and fungal ASVs, respectively.

Data analyses were conducted in R v.4.0 using the Phyloseq package v.1.32.0 (McMurdie and Holmes 2013) for α - and β -diversity metrics. Differences in α -diversity values (Chao 1, inverse Simpson's) were tested using generalized linear models in the car package v.3.0.8 (Fox et al. 2020) followed by Tukey's posthoc tests in the multcomp package v.1.4.13 (Hothorn et al. 2020). Multi-dimensional ordinations using principal coordinates analyses (PCoA) were performed on Bray-Curtis (Hellinger-transformed) dissimilarities to assess microbial community structure. To test whether seed microbial communities were significantly affected by environment or *B. napus* line, permutational multivariate analysis was carried out in the Vegan package v.2.5.6. (Oksanen et al. 2019) with the function adonis. Vegan was also used to obtain relative abundance of bacterial and fungal taxa. To identify taxa with differential abundance among environments, the analysis of composition of microbiomes (ANCOM) in QIIME2 was applied (Mandal et al. 2015).

We use the term "core microbiome" to describe the ASVs present in every *B. napus* line and replicate analyzed in this study and in

individual site years. Separate comparisons describe the taxonomy (class, order, family, and genus) of bacterial and fungal ASVs detected within a given site year. These comparisons highlight taxa that were common across different environments without necessarily being the exact ASV match.

Data deposition. Sequence data were deposited in the NCBI Sequence Read Archive, Bioproject ID PRJNA563687, accessions SAMN14541915 to SAMN14542010.

RESULTS

In total, 1,401,178 bacterial and 4,267,381 fungal reads were assigned to 3,000 and 1,765 ASVs, respectively (Table 2; Supplementary Fig. S2). α -Diversity estimators differed between 2016 and 2017 at the Saskatoon field location ($P < 0.05$). Specifically, bacterial and fungal diversity as well as bacterial richness were higher in 2016 (Fig. 1; Supplementary Table S1). Similarly, differences were detected between the three locations in 2017, where Melfort exhibited the highest richness and diversity for both bacterial and fungal microbial communities (Fig. 1; Supplementary Table S2). Comparisons within individual locations showed that *B. napus* line also significantly affected α diversity. For instance, in 2017 at Melfort and Saskatoon, line NAM 0 showed pronounced differences in fungal diversity when compared with other lines but the patterns were not consistent between locations or between years at the Saskatoon site (Fig. 1; Supplementary Tables S1 and S2).

PCoA plots based on Bray Curtis dissimilarity revealed a clear clustering of bacterial and fungal communities depending upon year (Fig. 2A and B) and location (Fig. 2C and D). Permutational multivariate analysis of bacterial and fungal communities corroborated that year and location (i.e., environmental conditions) ($P < 0.05$) were the main factors explaining the variation in the microbial community structure, with a significant interaction year $\times$ line and location $\times$ line for fungi but not for bacteria (Table 3). Thus, year explained 20% of the bacterial variance and 32% of the fungal variance. Similarly, location accounted for 34% of the bacterial variance and 43% of the fungal variance. Year and location interactions with line represented 18 and 26%, respectively, of the variance for fungal communities (Table 3). When all four site years were assessed separately, *B. napus* line accounted for 42 to 83% of the fungal variance within a given site year. Furthermore, Melfort 2017 was the only field where line did not explain bacterial community structure (Table 3).

Relative abundance of the predominant bacterial and fungal orders ($>1\%$) detected in the *B. napus* seed microbiome fluctuated between 2016 and 2017 in Saskatoon and among locations in 2017 (Fig. 3; Supplementary Tables S3 to S10). However, all site years had high relative abundance of Gammaproteobacteria orders

TABLE 2
Summary of amplicon sequence variants (ASVs) found in the seed microbiome of *Brassica napus* across years and locations

Microorganisms	All site years	Saskatoon 2016 and 2017	2017 all sites	Saskatoon 2016	Saskatoon 2017	Melfort 2017	Scott 2017
Bacteria							
Total	3,000	1,806	2,555	1,299	1,063	2,165	434
Core ^a	2	4	2	18	6	20	9
Fungi							
Total	1,765	1,082	1,425	619	675	901	308
Core ^a	11	25	11	32	26	19	30

^a ASVs found in every sample and replicate examined.

Enterobacteriales and Pseudomonadales and Dothideomycetes orders Pleosporales and Capnoidiales. Differential abundance analysis using ANCOM showed that members of the Enterobacteriales order substantially increased while Xanthomonadales and Entylomatales substantially decreased between 2016 and 2017 at the Saskatoon location (Supplementary Tables S3 and S4). Among the three locations sampled in 2017, Melfort showed a high relative abundance of members of the bacterial order Propionibacteriales (Supplementary Tables S5, S6, and S7). Similarly, Scott exhibited a higher relative abundance of members of the fungal

order Hypocreales whereas, at Melfort, the orders Tremellales and Sporidiobolales had higher relative abundance than at other locations (Supplementary Tables S8, S9, and S10).

Further analysis revealed the existence of a “core microbiome” present in every replicate, line, year, and location examined; this core included 2 bacterial and 11 fungal ASVs (Table 2). In addition, ASVs that were present in every sample within a site also belonged to common taxonomic groups across all site years. These included members of the bacterial orders Enterobacteriales, Pseudomonadales, Micrococcales, and Sphingomonadales as well as members

Fig. 1. α -Diversity measures of **A and B**, bacterial and **C and D**, fungal communities associated with canola seed harvested in Saskatchewan. NAM = nested association mapping population

of the fungal orders Pleosporales, Capnodiales, Tremellales, Filobasidiales, and Sporidiobolales (Table 4). More specifically, *Pseudomonas* spp., *Alternaria* spp., *Chalastospora gossypii*, *Mycosphaerella tassiana*, *Vishniacozyma victoriae*, *Dioszegia hungarica*, and *Stemphylium vesicarium* were found in all site years.

DISCUSSION

Environmental and genetic factors have been reported to shape the roots and rhizosphere microbiomes (Edwards et al. 2015; Liu et al. 2019; Simonin et al. 2020); however, their effect on other plant organs is poorly understood. Environmental conditions differ across sites and years as realized through contrasts in climate or weather as well as differences in soil type and resulting fertility. We analyzed the contribution of environment and genotype (line) in the *B. napus* seed-associated microbiota assemblage. Bacterial and fungal communities associated with eight *B. napus* lines were assessed in four different site years, 2 years in Saskatoon (2016 and 2017) and two additional locations in 2017 (Melfort and Scott). We confirmed that environment (i.e., site year) significantly affected bacterial and fungal community assemblages, reflecting that environmental conditions,

including biotic or abiotic components and weather, predominantly determine the seed microbiome. For example, precipitation was greater in 2016 than 2017 at the Saskatoon site, which may explain the differences in the seed microbial community structure, including richness and diversity indices (Allard et al. 2020; Mavrodi et al. 2018). Year-to-year differences in the seed microbiome were previously described in winter oilseed rape (Rocheffort et al. 2019) and radish (Rezki et al. 2018). In both studies, fungi were more affected by differences between years than bacteria, supporting our findings in spring *B. napus*.

Geographical location is known to shape the microbial composition of different plant organs in several crops (Barnett et al. 2015; Gdanetz and Trail 2017; Kim et al. 2018; Walters et al. 2018), including seed (Klaedtke et al. 2016; Walitang et al. 2018). Studies with bean (*Phaseolus vulgaris* L.) (Klaedtke et al. 2016) and barley (*Hordeum vulgare*) (Chen et al. 2016) confirmed that there is a site-specific effect linked to human practices and environmental conditions that determines microbial load and diversity in the seed microbiome. In the study carried out with barley seed, Chen et al. (2016) reported that harvesting method and harvesting date had a significant impact on the fungal community composition. Similar to our study, this work was carried out in Western Canada, including sites in Saskatchewan.

Fig. 2. Principal coordinate analysis (PCoA) based on Bray-Curtis index of bacterial and fungal amplicon sequence variants from canola seed harvested across **A and B**, 2 years and **C and D**, three locations in Saskatchewan. NAM = nested association mapping population.

Although the direct impact of soil type, pH or organic matter on the seed microbiota has not been described in the literature, findings in below- and aboveground microbiomes could give us an insight into how these properties influence seed. Previous reports suggest the presence of a communication network within plant organs, in which spermosphere, rhizosphere, and phyllosphere communities interact (Hassani et al. 2018; Lemanceau et al. 2017). Thus, microbial shifts in one organ will be reflected in the whole phytobiome. Soil type is characterized by different physicochemical properties that, together with root exudates, mediate the formation of unique and localized microenvironments and, consequently, microbial community structures (Berg and Smalla 2009; Lloyd et al. 2016). Experiments carried out in the Canadian Prairies by Lay et al. (2018) and Cordero et al. (2020) showed the effect of location and soil type on bacterial communities in canola roots and rhizosphere. Our study of the seed microbiome included fields in Dark Brown (Saskatoon and Scott) and Black (Melfort) soil zones that differed not only in soil texture but also in pH and organic matter content. These parameters could indirectly affect seed microbial assembly by causing differences in the soil microbiome which can act as an inoculum source for the seed (Nelson 2018). Among all soil characteristics, pH was documented in the literature as the key factor for bacterial community composition and diversity, being involved in numerous biogeochemical processes and, consequently, altering bacterial growth (Hartman et al. 2008; Lauber et al. 2009). Trials with maize (*Zea mays* L.) (Tan et al. 2020), soybean (*G. max*) (Zhang et al. 2018), wheat (*T. aestivum*) (Fan et al. 2018; Simonin et al. 2020), and canola (Cordero et al. 2020) showed that pH regulates microbial assemblage in rhizospheric and bulk soils. In our seed study, the Scott site was characterized by low pH (approximately 5). In contrast, Melfort was not only more alkaline (pH 6.4) than Scott but also had much higher organic matter content (8.2 versus 5.8%), which may have contributed to the highest richness and diversity levels found in both bacterial and fungal communities at Melfort. The correlation between organic matter content and

bacterial load in roots and aerial tissues was reported before by Haron et al. (2019), who indicated that variations in soil organic matter result in different plant-associated microbial communities.

Host genotype effect on the phytobiome assemblage has been examined before in oilseed crops, including soybean (Liu et al. 2019; Zhong et al. 2019), sunflower (*Helianthus annuus*) (Leff et al. 2017), olive (*Olea europaea* L.) (Mina et al. 2020), and canola (Taye et al. 2020); however, only two studies considered the effect of the host genotype on the *B. napus* seed microbiome. Endophytic and epiphytic studies with winter rapeseed revealed a cultivar-specific effect for both bacterial and fungal seed microbiomes (Rocheffort et al. 2019; Rybakova et al. 2017). When canola fields were assessed in our study, a higher plant line effect was observed in fungi than in bacteria. Our results are supported by a seed microbiome analysis carried out in different plants of the Brassicaceae family (Barret et al. 2015), where the host primarily affected the fungal community composition. In addition, the geographical location of the production region highly affects fungal assemblage (Barret et al. 2016). The lack of seed microbiome studies considering fungi impedes a deeper analysis of the host impact. According to Andreo-Jimenez et al. (2019), an active recruitment of fungal species by the plant host can occur to cope with or as a result of stress conditions; in some cases, fungi colonizes the plant to escape environmental stress.

Our results demonstrated the presence of microbial taxa shared between all lines and fields examined. *Pseudomonas* spp. as well as members of the Sphingomonadales order, previously reported as part of the core seed microbiome in *B. napus* (Links et al. 2014; Rocheffort et al. 2019), were among the bacterial taxa found in every single sample in our work. In wheat cultivars, *Pseudomonas* spp. were detected in several stages of plant development, from seed to leaf formation, suggesting that they play a critical role in plant fitness due to their ability to move systemically throughout the plant (Kuźniar et al. 2020). Furthermore, *Pseudomonas* and *Sphingomonas* strains isolated from rice (*Oryza sativa*) seed exhibited plant

TABLE 3
Permutational multivariate analysis of the microbial communities associated with *Brassica napus* lines

Site	Bacteria			Fungi		
	<i>F</i>	<i>R</i> ²	<i>P</i>	<i>F</i>	<i>R</i> ²	<i>P</i>
Saskatoon (2016 and 2107)						
Year	11.63	0.20	0.001	21.30	0.32	0.001
Line	1.22	0.18	0.093	1.30	0.19	0.102
Year × line	1.23	0.13	0.076	2.52	0.18	0.001
2017 (all sites)						
Location	18.00	0.34	0.001	25.87	0.43	0.001
Line	0.95	0.09	0.607	1.33	0.13	0.081
Location × line	1.13	0.14	0.172	4.86	0.26	0.001
Saskatoon 2016						
Line	1.41	0.38	0.012	1.64	0.42	0.001
Saskatoon 2017						
Line	1.45	0.39	0.003	3.95	0.63	0.001
Melfort 2017						
Line	1.04	0.31	0.414	11.52	0.83	0.001
Scott 2017						
Line	1.29	0.36	0.055	2.05	0.47	0.001

growth promotion traits, including siderophore production (Ruiza et al. 2011; Walitang et al. 2017). The *Dioszegia hungarica* fungus was also part of the core taxa. This Basidiomycota has been described in rice seed (Eyre et al. 2019), wheat seed (Nicolaisen et al. 2014), and wheat leaves (Ricks and Koide 2019) and is reported to be involved in suppression of plant pathogens (Hassani et al. 2018). *Alternaria* spp., *C. gossypii*, *M. tassiana*, and *S. vesicarium*, described in the literature as plant pathogens, were also identified here as part of the core seed microbiome. *Alternaria* spp. are known for causing dark spot disease on *Brassica* leaves (Kumar et al. 2014). Moreover, members of this genus are associated with spoilage and mycotoxin production (Bhat and Reddy 2017). Further investigation into relationships among organisms (e.g., between bacteria and fungi) can be explored to determine whether there are potential antagonistic interactions occurring that benefit plant health. For instance, Links et al. (2014) found *Pantoea* spp. in *T. aestivum* seed able to suppress *Alternaria* spp.

B. napus seed mainly contained members of the Enterobacteriales, Pseudomonadales, and Pleosporales bacterial orders (Barret et al. 2015; Links et al. 2014). Although Betaproteobacteriales has been reported as one of the major orders colonizing winter rapeseed (Rocheffort et al. 2019; Rybakova et al. 2017), we

did not find the same pattern on spring *B. napus*. Scott received the highest precipitation across all 2017 sites which, added to a delayed harvest, could explain the notable occurrence of the Hypocreales order (Paul et al. 2004). A high incidence of yeasts belonging to the Tremellales order was noticed in Melfort, possibly related to high soil organic matter content. Tremellales are basidiomycetes, which play a key role in the decomposition of organic matter (Banerjee et al. 2019; Ottesen et al. 2016). The observed shifts in microbial community composition across years and locations are driven by environment and host genotype. These shifts may also reflect plant fitness and resilience against biotic and abiotic stresses, which directly affect yield and productivity (Berg and Raaijmakers 2018; Singh and Trivedi 2017).

Acquisition, survival, and transmission of seed-associated microorganisms are determined by the mother plant (vertical transmission) as well as by agricultural management practices, weather, and seed storage conditions (horizontal transmission) (Barret et al. 2016; Singh and Mathur 2004). Our findings determined that environment and genotype influence the seed microbiome assemblage in spring *B. napus*. However, the environment appears to play a more significant role in shaping seed-associated microbial communities. Nevertheless, the existence of a core microbial taxa

Fig. 3. Relative abundance of the dominant **A**, bacterial and **B**, fungal orders in the seed microbiome of *Brassica napus* lines across years and locations.

TABLE 4
Taxonomic assignment of the bacterial and fungal amplicon sequence variants (ASVs) shared among all *Brassica napus* lines within a location^a

ASVs	Saskatoon 2016	Saskatoon 2017	Melfort 2017	Scott 2017
Bacteria				
Total	18	6	20	9
	Actinobacteria (2)	Bacillales, <i>Exiguobacterium</i> sp.	Bacillales, <i>Bacillus</i> sp. (2)	Acetobacteriales
	Bacillales, <i>Paenibacillus</i> sp.	Enterobacteriales (2)	Bacillales, <i>Exiguobacterium</i> sp.	Betaproteobacteriales, Burkholderiaceae
	Bacillales, <i>Saccharibacillus</i> sp.	Micrococcales	Betaproteobacteriales, Burkholderiaceae (2)	Betaproteobacteriales, <i>Ralstonia</i> sp.
	Betaproteobacteriales, Burkholderiaceae	Pseudomonadales, <i>Pseudomonas</i> sp.	Corynebacteriales, <i>Rhodococcus</i> sp.	Enterobacteriales
	Corynebacteriales, <i>Rhodococcus</i> sp.	Sphingomonadales	Cytophagales, <i>Hymenobacter</i> sp.	Enterobacteriales, <i>Erwinia</i> sp.
	Cytophagales, <i>Hymenobacter</i> sp. (2)	...	Enterobacteriales	Micrococcales
	Enterobacteriales	...	Micrococcales, Micrococcaceae (2)	Bacillales, <i>Paenibacillus</i> sp.
	Micrococcales (2)	...	Micrococcales, <i>Rathayibacter</i> sp.	Pseudomonadales, <i>Pseudomonas</i> sp.
	Micrococcales, <i>Rathayibacter</i> sp.	...	Propionibacteriales, <i>Micrococcus</i> sp.	Sphingomonadales
	Pseudomonadales, <i>Pseudomonas</i> sp.	...	Pseudomonadales, <i>Pseudomonas</i> sp. (4)	...
	Rhizobiales, Rhizobiaceae (2)	...	Rhizobiales, Rhizobiaceae	...
	Rhizobiales, <i>Methylobacterium</i> sp.	...	Rhizobiales, <i>Methylobacterium</i> sp.	...
	Rhodobacteriales, <i>Falsirhodobacter</i> sp.	...	Rhodobacteriales, <i>Falsirhodobacter</i> sp.	...
	Sphingomonadales	...	Sphingomonadales	...
Fungi				
Total	32	26	19	30
	Capnodiales	Agaricostilbales, <i>Kondoa sorbi</i>	Capnodiales	Capnodiales
	Capnodiales, <i>Mycosphaerella tassiana</i> (2)	Capnodiales	Capnodiales, <i>Mycosphaerella tassiana</i> (2)	Capnodiales, <i>Mycosphaerella tassiana</i>
	Entylomatales (2)	Capnodiales, <i>Mycosphaerella tassiana</i> (2)	Cystofilobasidiales, <i>Cystofilobasidium macerans</i> (2)	Cystofilobasidiales, <i>Cystofilobasidium macerans</i> (3)
	Entylomatales, <i>Tilletiopsis washingtonensis</i>	Entylomatales, <i>Tilletiopsis washingtonensis</i>	Dothideales, <i>Aureobasidium pullulans</i>	Filobasidiales, <i>Filobasidium</i> sp. (2)
	Filobasidiales, <i>Filobasidium</i> sp.	Filobasidiales, <i>Filobasidium</i> sp.	Filobasidiales, <i>Filobasidium chernovii</i>	Filobasidiales, <i>Filobasidium chernovii</i>
	Filobasidiales, <i>Filobasidium magnum</i>	Filobasidiales, <i>Filobasidium magnum</i>	Filobasidiales, <i>Filobasidium magnum</i>	Filobasidiales, <i>Filobasidium magnum</i>
	Hypocreales, Nectriaceae	Hypocreales, <i>Fusarium</i> sp.	Holtermanniales, <i>Holtermanniella takashimae</i>	Holtermanniales, <i>Holtermanniella takashimae</i> (2)
	Hypocreales, <i>Fusarium</i> sp.	Pleosporales, <i>Alternaria</i> sp.	Pleosporales, <i>Alternaria</i> sp.	Hypocreales, <i>Fusarium</i> sp.
	Hypocreales, <i>Sarocladium strictum</i>	Pleosporales, <i>Alternaria brassicae</i>	Pleosporales, <i>Chalastospora gossypii</i>	Hypocreales, <i>Trichothecium roseum</i> (2)
	Pleosporales, <i>Alternaria</i> sp.	Pleosporales, <i>Chalastospora gossypii</i> (4)	Pleosporales, <i>Stemphylium vesicarium</i>	Pleosporales, Didymellaceae
	Pleosporales, <i>Alternaria brassicae</i>	Pleosporales, Leptosphaeriaceae	Sporidiobolales, <i>Rhodotorula</i> sp.	Pleosporales, <i>Alternaria</i> sp.
	Pleosporales, <i>Alternaria obovoidea</i>	Pleosporales, <i>Paradendryphiella arenariae</i>	Sporidiobolales, <i>Sporobolomyces ruberrimus</i>	Pleosporales, <i>Alternaria brassicae</i>

(Continued on next page)

^a Taxa in bold denote members of the “core microbiome” found across locations. Total = number of bacterial and fungal ASVs found in every sample and replicate examined (per site).

TABLE 4 (Continued from previous page)

ASVs	Saskatoon 2016	Saskatoon 2017	Melfort 2017	Scott 2017
	Pleosporales, <i>Chalostopora gossypii</i> (4)	Pleosporales, <i>Stemphylium vesicarium</i>	Tremellales, <i>Dioszegia hungarica</i>	Pleosporales, <i>Alternaria subcucurbitae</i>
	Pleosporales, Leptosphaeriaceae	Sordariomycetes	Tremellales, <i>Vishniacozyma</i> sp.	Pleosporales, <i>Chalostopora gossypii</i> (2)
	Pleosporales, <i>Paradendryphiella arenariae</i>	Sporidiobolales, <i>Sporobolomyces roseus</i>	Tremellales, <i>Vishniacozyma victoriae</i> (3)	Pleosporales, <i>Paradendryphiella arenariae</i>
	Pleosporales, <i>Stemphylium vesicarium</i>	Tremellales, <i>Bulleromyces</i> sp.	...	Pleosporales, <i>Stemphylium vesicarium</i>
	Sordariomycetes	Tremellales, <i>Dioszegia hungarica</i>	...	Sporidiobolales, <i>Rhodotorula</i> sp.
	Sporidiobolales, <i>Sporobolomyces roseus</i>	Tremellales, <i>Vishniacozyma</i> sp.	...	Sporidiobolales, <i>Sporobolomyces roseus</i>
	Sporidiobolales, <i>Sporobolomyces ruberrimus</i>	Tremellales, <i>Vishniacozyma victoriae</i> (3)	...	Tremellales, <i>Bulleromyces</i> sp.
	Tremellales, <i>Bulleromyces</i> sp.	Tremellales, <i>Vishniacozyma carnescens</i>	...	Tremellales, <i>Dioszegia hungarica</i>
	Tremellales, <i>Vishniacozyma</i> sp.	...	...	Tremellales, <i>Vishniacozyma</i> sp.
	Tremellales, <i>Dioszegia hungarica</i>	...	...	Tremellales, <i>Vishniacozyma victoriae</i> (3)
	Tremellales, <i>Vishniacozyma carnescens</i>	...	...	...
	Tremellales, <i>Vishniacozyma victoriae</i> (4)	...	...	...

implies that *B. napus* plants recruit and carry bacterial and fungal species that could interact with further generations, affecting plant fitness in novel environments. Further research should consider the impacts of plant genotype × environment interactions on the entire plant microbiome (i.e., the phytobiome), with special focus on optimization in plant breeding.

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